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Reticulate evolution in nuclear Middle America causes discordance in the phylogeny of palm-pitvipers (Viperidae: *Bothriechis*)

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Abstract

Aim: A number of processes can lead to weak or conflicting phylogenetic signals, especially in geographically dynamic regions where unstable landscapes and climates promote complex evolutionary histories. The Middle American pitviper genus *Bothriechis* has a complex biogeographic distribution and previous phylogenetic analyses have recovered conflicting topologies based on the data type used. Here, we tested whether historic conflicts in the phylogeny were the result of reticulate evolution and whether the inferred biogeographic history of the group would enable contact among reticulate lineages.

Location: Middle America.

Taxon: Palm-pitvipers (genus *Bothriechis*).

Methods: We generated a phylogenomic dataset using an anchored phylogenomics approach and inferred a genomics-based species tree and mitochondrial tree to assess incongruence among datasets. We then generated a dated phylogeny and conducted ancestral area reconstruction to examine the biogeographic history surrounding the diversification of these species. We additionally tested whether the discordance among trees is better explained by lineage sorting or reticulate evolution by testing models of reticulate evolution inferred through multiple methods.

Results: We found strong support for discordance in the phylogeny of *Bothriechis* and corresponding evidence for reticulate evolution among lineages with incongruent placement. Ancestral area reconstruction placed these taxa in adjacent regions during the time period when reticulation was projected to take place and suggested a biogeographic history heavily influenced by vicariant processes.

Main conclusions: Reticulation among geographically proximate lineages has driven apparent genomic discordance in *Bothriechis* and is responsible for historical incongruence in the phylogeny. Inference of the order of events suggests that reticulation among nuclear Middle American taxa occurred during a time of geologic upheaval, promoting lineage divergence and secondary contact. Reticulate evolution and similar processes can have substantial impacts on the evolutionary trajectory of taxa and are important to explicitly test for in biogeographically complex regions.

1 | INTRODUCTION

A variety of systematic and inherent biases can contribute to phylogenetic instability and discordance (Funk & Omland, 2003; Jeffroy, Brinkmann, Delsuc, & Philippe, 2006; Pamilo & Nei, 1988). The use of next-generation sequencing and genome-scale datasets were expected to provide resolution for recalcitrant nodes, and, while genomic datasets have resolved some relationships (Arcila et al., 2017; Breinholt et al., 2017), many systems remain challenged by discordance and conflicting phylogenetic signals (Smith, Moore, Brown, & Yang, 2015).

Many processes can lead to weak phylogenetic signal or discordance (Doyle, 1992; Giarla & Esselstyn, 2015; Maddison, 1997; Rieseberg, Baird, & Gardner, 2000), especially in historically dynamic geographic regions where unstable landscapes and climate promote complex evolutionary histories (Boissin, Stöhr, & Chenuil, 2011; Daza, Smith, Páez, & Parkinson, 2009; Ornelas et al., 2013). Changing landscapes can create novel habitats and open niches allowing for rapid radiations where the order of diversification can be difficult or impossible to determine (Giarla & Esselstyn, 2015; Weissing, Edelaar, & Van Doorn, 2011). Similarly, disjunct habitats such as island archipelagos and isolated montane "sky-islands" can lead to intricate patterns of speciation through vicariance or dispersal (Juan, Emerson, Oromí, & Hewitt, 2000; Savage, 1982; Zaher et al., 2018).

While many geographic regions have complicated histories, few are as biologically rich and geologically complex as Middle America. Middle America lies at the junction of several tectonic blocks, whose movement and the associated volcanic activity have produced a diverse topographic landscape (MacMillan, Gans, & Alvarado, 2004; Marshall, 2007; Townsend, 2014). The formation of Middle American cordilleras and volcanic sky islands has in turn promoted complex patterns of diversification in a number of taxa. In these habitats, connectivity and secondary gene flow often erode phylogenetic signal or introduce gene tree discordance (Rieseberg et al., 2000; Sardell, Albert, & Uy, 2016; Thom et al., 2018). Thus, when examining evolution in regions with complex climatic or geologic histories where several evolutionary processes affect speciation simultaneously or in succession, many hypotheses may require evaluation to identify and explain sources of discordance and/or conflicting signal.

Phylogenetic discordance among data types may indicate a role of historic gene flow in shaping evolutionary histories. If gene flow is sufficiently high between ancestral lineages, a group's phylogeny may be considered reticulate such that it can no longer be characterized by strictly bifurcating processes. However, inferring trees for groups with gene flow can be problematic as multispecies coalescent methods that account for discordance due to incomplete lineage

sorting (ILS) can fail in the face of moderate gene flow (Leaché, Harris, Rannala, & Yang, 2014). To this end, several methods have been proposed to model reticulate evolution among lineages, some of which can simultaneously account for ILS (Pickrell & Pritchard, 2012; Solís-Lemus & Ané, 2016; Than, Ruths, & Nakhleh, 2008; Yu & Nakhleh, 2015). These methods offer an opportunity to explicitly test introgression as a source of discordance by comparing optimality criteria, as well as infer a phylogenetic network that can more accurately reflect a group's history. Moreover, phylogenomic datasets using hundreds of loci from across the genome provide a robust input for reticulate inference, lending confidence to network assessment in the face of discordance (Solís-Lemus & Ané, 2016).

One Middle American group with a complex biogeographic distribution and disputed evolutionary history is the pitviper genus *Bothriechis*. This clade of 11 species is mainly restricted to mid-to-high elevation habits, which has led to a mosaic of species distributions across Middle American montane habitats (Campbell & Lamar, 2004) (Figure 1). Speciation in this group is thought to have progressed largely allopatrically as montane populations were separated by changing topologies or other biogeographic breaks (Doan, Mason, Castoe, Sasa, & Parkinson, 2016). For instance, the Nicaraguan Depression has been implicated as a biogeographic break separating the nuclear Middle American species (*Bothriechis aurifer, B. bicolor, B. guifarroi, B. marchi, B. rowleyi* and *B. thalassinus*) from those species restricted to the southern Middle American Isthmus (*B. lateralis, B. nigroviridis, B. nubestris* and *B. supraciliaris*) (Castoe et al., 2009; Daza, Castoe, & Parkinson, 2010). Similarly, the Motagua–Polochic fault has been proposed as a break dividing *B. aurifer, B. bicolor* and *B. rowleyi* from *B. guifarroi, B. marchi* and *B. thalassinus* (Castoe et al., 2009; Daza et al., 2010). However, within and among these groups the order of diversification is questionable based on historic incongruences in inferred phylogenies.

The earliest phylogeny of *Bothriechis* was based on morphology and allozyme data (Crother, Campbell, & Hillis, 1992) and suggested a complex biogeographic history based on a recovered clade containing both nuclear Middle American and southern Middle American taxa (Figure 1a). Later, the incorporation of mitochondrial data resulted in the recovery of an incongruent topology (Taggart, Crother, & White, 2001), with a monophyletic nuclear Middle American clade (Figure 1b). Subsequent phylogenies supported this, but were similarly driven by mitochondrial sequence data. It is therefore unclear whether the incongruences in these results are due to differences in analytical approach and total sequence data, or if they reflect biological discordance in the system. Additionally, the description of several new species, some of which add biogeographic–phylogenetic conflict (Figure 1c), further call into question the evolutionary processes shaping this
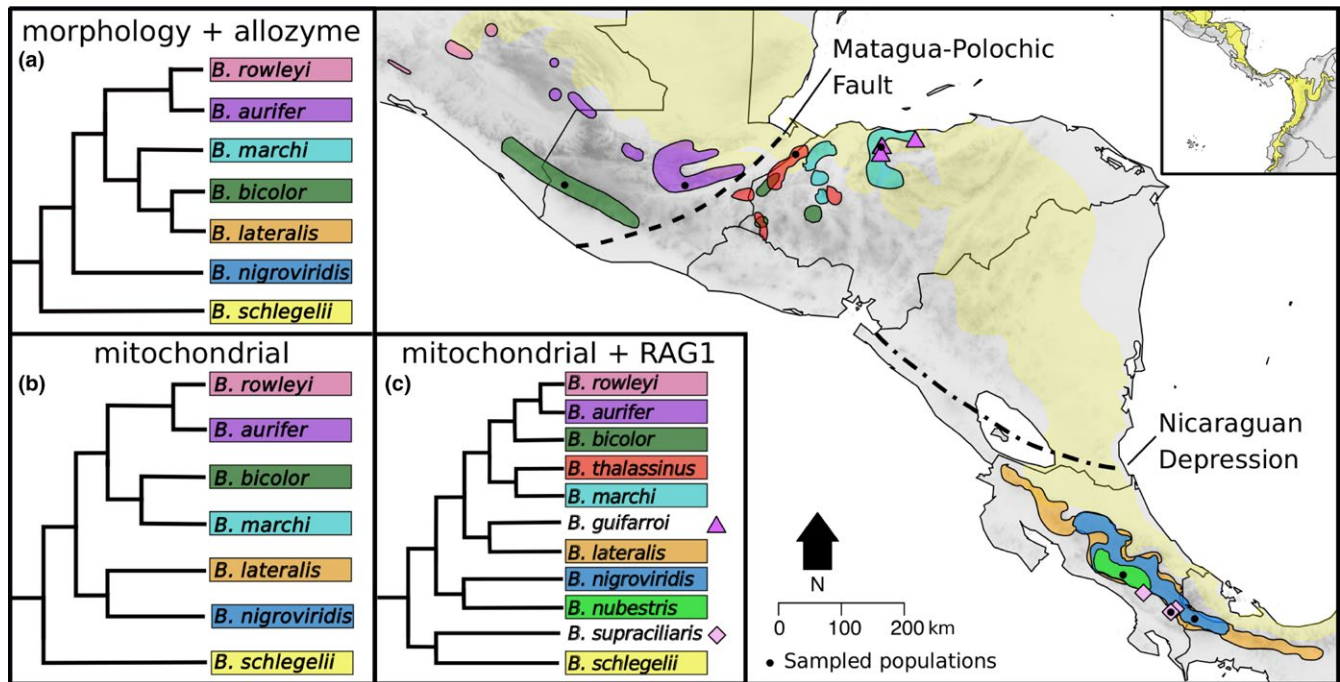
FIGURE 1 Phylogenetic hypotheses (a, b, c) for the evolution of *Bothriechis* in relation to their geographic to distributions. (a) Phylogeny of Crother et al. (1992) based on morphological and allozyme characters. Notably, *B. lateralis* is nested with northern Middle American taxa suggesting a southward dispersion of this taxon to Costa Rica. (b) Phylogeny of Taggart et al. (2001) based on 12S sequences with a monophyletic northern Middle American clade suggesting a northward invasion. (c) Phylogeny of Doan et al. (2016) including all currently described species. Ranges are based on Campbell and Lamar (2004); Townsend et al. (2013); and Doan et al. (2016). Species with limited or poorly sample ranges shown as points and sampled populations are denoted with a black dot where known [Colour figure can be viewed at wileyonlinelibrary.com]

group (Campbell & Smith, 2000; Doan et al., 2016; Solórzano, Gómez, Monge-Nájera, & Crother, 1998; Townsend, Medina-Flores, Wilson, Jadin, & Austin, 2013).

The historical incongruences and growing evidence of a complex evolutionary history in *Bothriechis* make it an ideal group to test for discordance among data types and test for specific processes generating discordance in a biogeographic context. Here, we generated a phylogenomic dataset using an anchored phylogenomics approach (Lemmon, Emme, & Lemmon, 2012) and inferred a genomics-based species tree and mitochondrial tree to assess incongruence among phylogenomic and mitochondrial datasets. We then generated a dated phylogeny and conducted ancestral area reconstruction to examine the biogeographic history surrounding diversification of these species. Finally, we tested whether the discordance among trees is better explained by lineage sorting or reticulate evolution. Specifically, we used a model testing approach to compare models of reticulate evolution inferred through multiple methods to strictly bifurcating trees.

2 | MATERIALS AND METHODS

2.1 | Sampling and sequence generation

We collected 17 tissue samples representing the 11 recognized species of *Bothriechis*, with six species (*B. aurifer, B. bicolor, B. lateralis, B.*

nubestris, B. schlegelii and *B. supraciliaris*) represented by two samples. We additionally included sampling from nine outgroup taxa representing each genus of Middle American viper, as well as *Crotalus cerastes* and *Agkistrodon contortrix* as representatives of the Northern American pitvipers. For biogeographic analyses, *Bitis nasicornis*, *Pareas margaritophorus*, *Bungarus multicinctus*, *Causus maculatus* and *Oxybelis aeneus* were also included for tree calibration. Data for sampled taxa are available in Appendix S1, Table S1 in Supporting Information.

We used the Anchored Hybrid Enrichment method described by Lemmon et al. (2012) with probe kit improvements for squamate taxa described by Ruane, Raxworthy, Lemmon, Lemmon, and Burbrink (2015) and Tucker et al. (2016) to generate genomic sequences for phylogenomic analyses (additional details in Appendix S2). Libraries were sequenced on a HiSeq 2500 at the FSU College of Medicine's Translational Science Laboratory and approximately 1 Gb of 150 bp pair-end sequence data were collected per sample.

2.2 | Data processing

We processed the data following the bioinformatics pipeline of Breinholt et al., 2017 (additional details in Appendix S2). Briefly, we cleaned raw reads using TRIM GALORE! 0.4.4 (Krueger, 2015) and assembled anchored loci using the IBA.py script (Breinholt et al., 2017). Assembled sequences for each individual were reorganized by locus and aligned to the probe region with MAFFT 7.035b (Katoh &

Standley, 2013). To determine orthology of trimmed probe regions, we mapped sequences to the Burmese python genome with NCBI BLASTN 2.7.1 (Camacho et al., 2009), filtered BLAST results by bit score and selected single hit sequences mapped to the same region of the python genome.

Orthologous copies for each taxon were then aligned with MAFFT and FASCONCAT-G 1.04 (Kück & Longo, 2014) was used to generate strict consensus sequences for taxa with multiple isoforms. Duplicates were removed and the remaining sequences were aligned using MAFFT to generate the final alignment set for each locus. Final alignments were trimmed by density and entropy using Trim_DE.py (Breinholt et al., 2017) to remove sites with a density of <0.6% or entropy >1.5 and were checked manually in GENEIOUS 10.2.3 (https://www.geneious.com, Kearse et al., 2012).

2.3 | Species tree estimation

We used concatenation and species tree approaches in phylogenetic estimation to assess potential conflict in phylogenetic signal and ensure robust inferences. Both concatenation and species tree estimation were carried out using the Palmetto high performance computing cluster at Clemson University. For concatenated analyses, alignments for all loci were first concatenated in GENEIOUS. We then determined the most appropriate data partitioning scheme and model of nucleotide substitution using PARTITIONFINDER2 2.1.1 (Lanfear, Frandsen, Wright, Senfeld, & Calcott, 2016), specifying the relaxed hierarchical clustering algorithm (rclust) and AICc as the optimality criterion. We obtained our best-tree estimate in RAxML 8.2.11 (Stamatakis, 2014) using 100 independent searches and a GTRGAMMA model for each partition. Nodal support values were calculated by performing 1,000 bootstrap replicates that were mapped to the best tree. To assess the degree of incongruence at internal nodes we calculated internode certainty (IC), tree certainty (TC) and relative tree certainty (RTC) based on trees estimated for each locus using RAxML with independent GTRGAMMA models and 100 independent searches.

Coalescent and coalescent-based inference of the species tree was conducted using *BEAST2 0.13.5 (Ogilvie, Bouckaert, & Drummond, 2017) and ASTRAL III 5.6.1, respectively (Mirarab et al., 2014; Zhang, Sayyari, & Mirarab, 2017). For *BEAST2 analyses, we followed Leaché, Wagner, et al. (2014) in selecting the 20 loci with the highest number of parsimony informative sites. Site models, clock models and trees were unlinked across all loci and each locus was assigned an uncorrelated lognormal clock and appropriate substitution model determined in PARTITIONFINDER2. The tree prior was set to Yule with a linear with constant root population size parameter. We ran *BEAST four independent times for 1.5 billion generations and assessed convergence among runs in TRACER 1.6.0 (Rambaut, Suchard, & Drummond, 2015). For ASTRAL analyses we used the gene trees to assess internal node incongruence with nodal support estimated with 1,000 bootstrap replicates. Gene trees for each locus were then used as input for ASTRAL to estimate the species tree. To better assess gene tree discordance across

nodes in the tree, we annotated quartet support for each node by specifying the -t 8 option. The resulting species trees for concatenated and coalescent analyses were visualized using FIGTREE 1.4.3 (Rambaut, 2012).

2.4 | Mitochondrial genome analyses

We extracted mitochondrial sequence data captured as a by-product of the anchored phylogenomics workflow and conducted mitochondrial genome-based analyses. We inferred mitochondrial genomes for all specimens of *Bothriechis* and representatives of Middle American genera by mapping trimmed readsets to the *Bothrops jararaca* mitochondrial genome (GenBank accession: NC030760, Dantas Almeida et al., 2016) in GENEIOUS with a minimum read coverage of 5x.

Sequences for each of the 13 protein coding loci and the 12S and 16S ribosomal RNAs were extracted from each individual and a partitioning scheme and nucleotide substitution model for each locus determined by PARTITIONFINDER2. We then inferred a gene tree for each locus using RAxML with 1,000 bootstrap replicates. Gene trees were checked individually for their support for the species tree or an alternate hypothesis. For a mitogenome-wide approach, we used HOMBLOCKS 1.1.1 (Bi, Mao, Xing, & Cao, 2017) to construct a reduced, phylogenetically informative multiple sequence alignment based on locally colinear synteny blocks of the mitochondrial genome. The HOMBLOCKS alignment was then used to infer a mitochondrial phylogeny in RAxML using a GTRGAMMA model based on PARTITIONFINDER2 output of HOMBLOCKS with 1,000 bootstrap replicates.

2.5 | Phylogenetic dating

To examine the historical biogeography underlying the speciation of *Bothriechis*, we produced a dated phylogeny using MCMCTREE in PAML 4.9 (Yang, 2007). For MCMCTREE analyses, we used the North American Crotalinae dataset and additionally included five outgroup taxa as nodal-calibration points. The reference topology used for MCMCTREE was based on ML analyses of the concatenated anchored dataset. Four calibration points were used to calibrate the clock (see Appendix S3) and estimates of branch lengths were obtained using BASEML with the GTR+G model. The autocorrelated rates model was used to set the rate prior on internal nodes. MCMCTREE was run for 5,000,000 iterations as burn-in and then sampled every 1,000 generations to collect 50,000 samples. MCMCTREE was run three times with different random seeds and checked for convergence.

2.6 | Biogeographic inference

To assess the historical biogeography of *Bothriechis*, we used the recovered time calibrated phylogeny and estimated ancestral areas of each species with the R package 'BioGeoBEARS' (Matzke, 2013). We first pruned the dated tree to single representatives of only *Bothriechis* species using the droptip function of the R package 'APE' (Paradis, Claude, & Strimmer, 2004). We then assigned each *Bothriechis* species

to a biogeographic distribution based on their occupation of four biogeographic regions, which correspond to known biogeographic breaks in Middle America (see Appendix S3). We modelled differential dispersion probability across these regions with three categories based on likelihood of dispersal (0.1—unlikely, 0.5—moderately likely, 1—very likely) as in Feng et al. (2017). Assignment to one or more biogeographic regions was determined based on verified, georeferenced specimens on VertNet and positively identified species on iNaturalist. To infer ancestral areas, we evaluated the DEC, DIVA and BAYAREA models based on AIC. We used the best model to visualize the conditional probabilities of ancestral *Bothriechis* occupying various biogeographic ranges and compared the most likely occupation of ancestors surrounding discordant nodes.

2.7 | Reticulation analyses

We tested the hypothesis that recent and/or historic gene flow has led to conflicting phylogenetic signals in *Bothriechis* using three programs PHYLONET 3.6.2 (Than et al., 2008), SNAQ implemented in the Julia package 'PhyloNetworks' (Solís-Lemus & Ané, 2016), and TREEMIX 1.13 (Pickrell & Pritchard, 2012). PHYLONET and SNAQ infer phylogenetic networks using sequence data, gene trees or 4-taxon concordance factors while TREEMIX uses a statistical algorithm incorporating allele frequency data to determine the most likely tree with a specified number of migration events (i.e. gene flow) (Pickrell & Pritchard, 2012). Additional details on these analyses are available in Appendix S2.

To evaluate the relative contribution of reticulation events, we inferred networks and trees with 0–9 reticulations for each method, which also served as explicit tests of whether ILS alone sufficiently explains gene tree discordance. We calculated AIC for each model based on log likelihood or log pseudo-likelihood score treating each reticulation as a free parameter in the model and determined the optimal number of reticulations based on the relative change in AIC. Where models with one or more reticulations performed better, we concluded that ILS alone insufficiently explained observed gene tree discordance.

All PHYLONET and SNAQ analyses used gene trees inferred for each anchored locus in RAxML as in the species tree estimation analyses (above). To implement TREEMIX, we phased alleles for each sample using bash and python wrappers for BWA 0.7.16 (Li & Durbin, 2009) and GATK 3.8.1 (McKenna et al., 2010) based on scripts from (Alexander, 2015) and extracted independent SNPs from each locus. For PHYLONET analyses, we used the maximum pseudo-likelihood criterion for network selection (Yu & Nakhleh, 2015) and performed 25 iterations of 500 independent searches of network space retaining the top five models in each iteration. For SNAQ analyses we conducted 500 searches of network space and retained the network with the highest pseudolikelihood as the best network. We used the ASTRAL topology as the starting tree for the first network search, with the resulting best networks as starting networks for subsequent searches with additional reticulation edges. TREEMIX was run with each *Bothriechis* species defining a population and specifying 0–9 migration edges and we used the three-population and four-population tests to calculate the *f3* and *f4* statistics for all population combinations. Finally, to assess the number and signal of informative SNPs across all loci, we calculated Patterson's *D*-statistics for all population combinations with an alignment of SNPs from all loci with all terminals using the R package 'evobiR' (Blackmon & Adams, 2015).

3 | RESULTS

3.1 | Bioinformatic processing

After sequencing we obtained an average of 3,148,191 bp pair-end reads per sample (range 316,456–6,178,333) (see Appendix S4, Table S2). Bioinformatic processing recovered 405 anchored loci for phylogenetic inference, the most extensive dataset to date for phylogenomic analysis of palm-pitvipers. The average alignment length per locus was 668 bp (range 434–1,185). The final concatenated alignment consisted of 269,957 base pairs.

3.2 | Species tree analyses

Both species tree and concatenation methods of analysis recovered trees with strong support and largely consistent topologies (Figure 2, see Figure S1 and Figure S2 in Appendix S5). Though the relationships among genera, were not fully resolved, *Bothriechis* was recovered as monophyletic in all analyses. The clade composed by *B. schlegelii* and *B. supraciliaris* was found as the most basal lineage. The clade formed by *B. nigroviridis* and *B. nubestris* was sister to a clade containing the nuclear Middle American *Bothriechis* and *B. lateralis*, reflecting an early divergence of this morphologically distinct lineage. *Bothriechis aurifer*, *B. bicolor*, and *B. rowleyi* were recovered as a strongly supported clade, though their order of diversification varied among methods and was comparatively poorly supported. ASTRAL triplet scores and *BEAST analyses for this node showed similar support for each of the three possible topologies among gene trees. The lack of congruence in the order of diversification of these taxa may reflect a lack of power in the dataset for resolving this node, or a high degree of ILS as a result of a rapid speciation of these three taxa.

Among both concatenation and species tree analyses of anchored loci, *B. marchi*+*B. thalassinus* formed a clade with *B. lateralis*+*B. guifarroi*. This topology disagrees with the relationship recovered with morphological and allozyme data—which place *B. lateralis* sister to *B. bicolor* (Crother et al., 1992), and also contradicts the relationships observed from later phylogenies placing *B. marchi*+*B. thalassinus* with the *B. aurifer*+*B. bicolor*+*B. rowleyi* clade. The ASTRAL triplet scores for this node were inconsistent with what would be expected under strict lineage sorting (e.g. strong support for one topology, and lower but approximately equal support for the other two) (see Figure S2 in Appendix S5). This suggests that processes other than strict ILS are impacting the relationships of taxa at this node.

3.3 | Mitochondrial analyses

Mitochondrial genome coverage averaged 95.5% per sample (range 85.8%–98.7%). Mitochondrial sequence accession numbers and
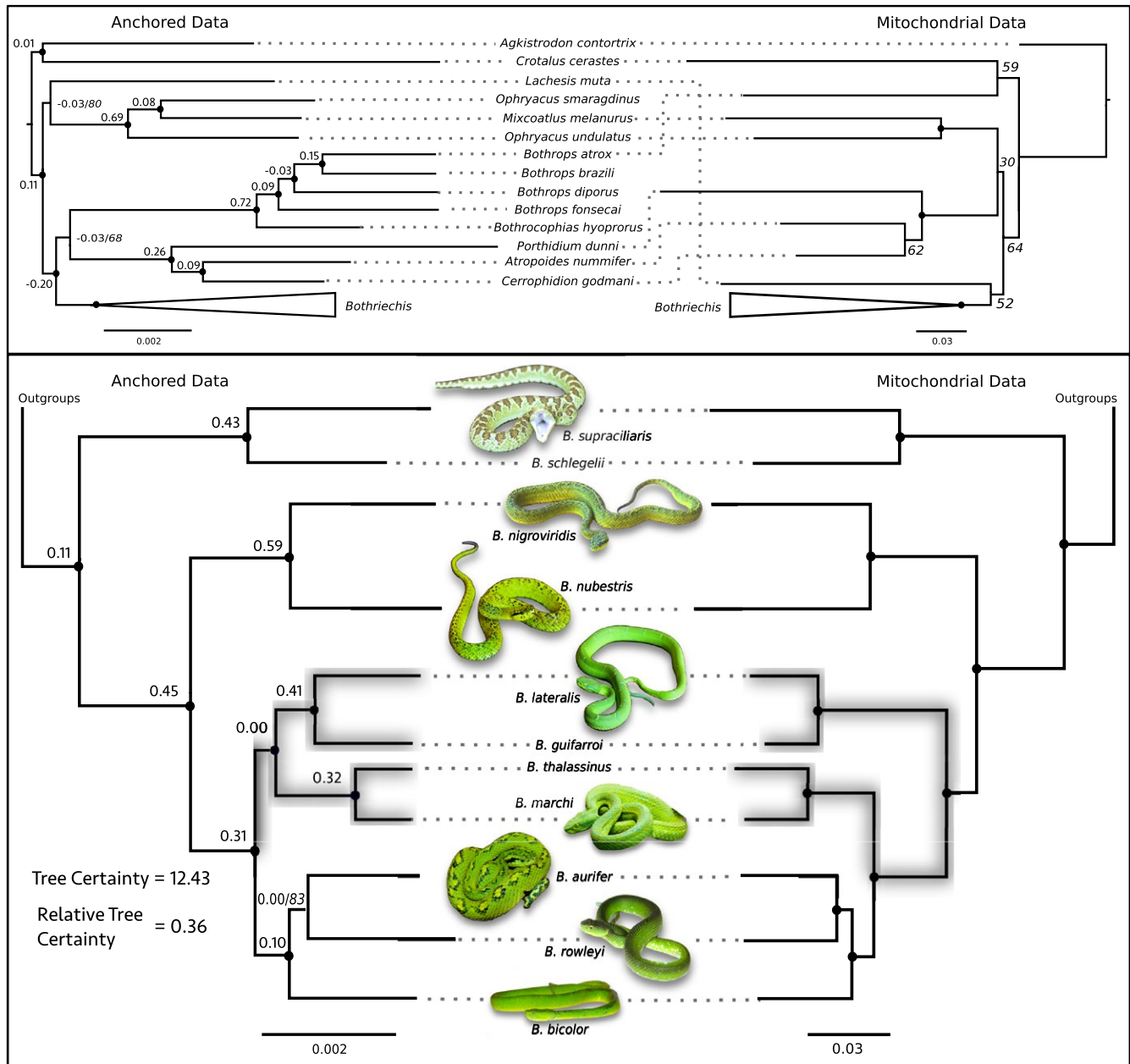
FIGURE 2 Concatenated anchored loci phylogeny (left) and mitochondrial phylogeny (right) of New World pitviper genera and *Bothriechis* species. Values by nodes show internode certainty and bootstrap support below 95 (italics). Dots on nodes indicate bootstrap support above 95. Discrepancies in the interspecific relationships of *Bothriechis* when using anchored loci versus mitochondrial data are shaded [Colour figure can be viewed at wileyonlinelibrary.com]

recovered lengths are given in Tables S1 and S2 and in Appendices S1 and S4 respectively.

The interspecific relationships within *Bothriechis* were strongly supported and most relationships were consistent with those of the anchored loci analyses. However, in the mitochondrial tree, the clade *B. marchi*+*B. thalassinus* was found as sister to the clade composed by *B. aurifer*, *B. bicolor* and *B. rowleyi* instead of the clade *B. lateralis*+*B. guifarroi* (shaded branches in Figure 2). Single mitochondrial gene phylogenies lacked the resolution of the HOMBLOCKS alignment, but similarly supported this conflicting relationship (Figure S3 in Appendix S4). This topology is consistent with other mitochondrially

driven phylogenies (Castoe et al., 2009; Daza et al., 2010; Doan et al., 2016; Townsend et al., 2013).

3.4 | Dating and biogeographic inference

Results of time calibration analyses were generally consistent with those of recent work (Alencar et al., 2016). We inferred that the common ancestor of *Bothriechis* arose approximately 18 Ma, with many instances of diversification occurring in the late Miocene–early Pliocene (Figure 3, see Figure S4 in Appendix S5). The *B. nigroviridis*+*B. nubestris* lineage was found to have diverged from the
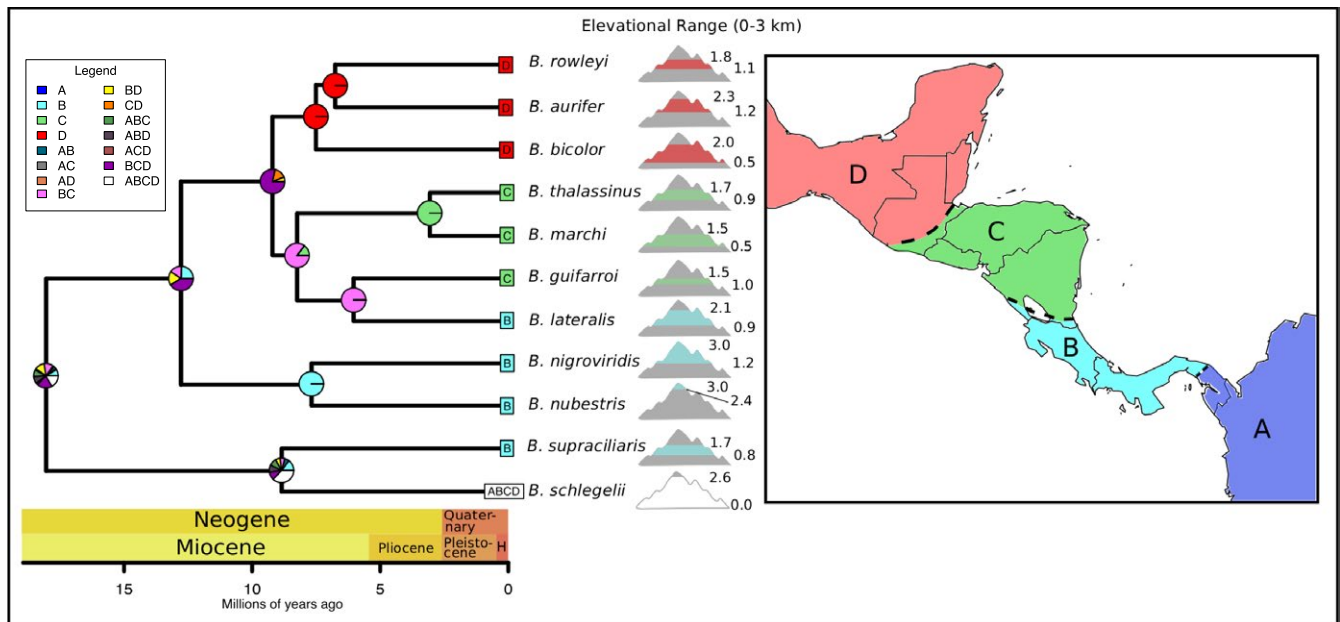
FIGURE 3 Ancestral area reconstruction from 'BioGeoBEARS' of *Bothriechis*. Piecharts on nodes indicate likelihood of originating in a given region. Biogeographic regions roughly correspond with South America (A; dark blue), the lower Middle American isthmus (B: light blue), the Chortís block region (C: green), and the Mayan block region (D: red). Mountain icons in the center of the figure indicate the elevational distributions of each species in kilometers above sea level [Colour figure can be viewed at wileyonlinelibrary.com]

other *Bothriechis* approximately 7.5–13 Ma, which may have been a result of a shift to higher elevation habitats. Divergence of a Mayan block clade (*B. aurifer*, *B. bicolor* and *B. rowleyi*) and a largely Chortís block clade (*B. guifarroi*, *B. lateralis*, *B. marchi* and *B. thalassinus*) occurred approximately 8.5–10 Ma, concordant with the timing of the west to east progression of the Chortís block along the Mayan block to its current position (Rogers, Mann, & Emmet, 2007; Townsend, 2014). The divergence of the *B. guifarroi*+*B. lateralis* and the *B. marchi*+*B. thalassinus* lineages likely occurred shortly thereafter while speciation of *B. aurifer*, *B. bicolor* and *B. rowleyi* likely occurred in a contracted time period between 7–9 Ma.

Model evaluation in 'BioGeoBEARS' found DEC as the most probable model (see Table S3 in Appendix S5). Ancestral area reconstruction of *Bothriechis* showed low confidence in estimating the distribution of the common ancestor of *Bothriechis* but indicated the ancestor of *B. schlegelii* and *B. supraciliaris* likely inhabited all of Middle America while the common ancestor of the remaining taxa was restricted to the region above the Isthmus of Panama (Figure 3). The primarily northern Middle American clade likely retained this distribution, prior to splitting into the Mayan block and Chortís block+Southern Middle American clades. Most species in this latter group later became restricted to the Chortís block, while *Bothriechis lateralis*' occupation of Southern Middle America was found to be the result of vicariant processes.

3.5 | Reticulation analyses

Reticulation analyses in all three methods supported a role for historical gene flow in the diversification of *Bothriechis*. Trees recovered by specifying zero reticulation events (i.e. bifurcating trees)

were consistent with those recovered using concatenation and species tree approaches, thus establishing an appropriate baseline for evaluating the effect of adding reticulation. Due to computational limitations we were only able to effectively evaluate networks with one or two reticulation events in PHYLONET and networks with more than five reticulations were not recovered by SNaQ. Overall, model comparisons of species tree and reticulation models supported a model of two reticulation events (Figure S5 in Appendix S5), which performed better than the species tree in all three methods.

The top networks with two reticulation events inferred by PHYLONET, SNaQ and TREEMIX are shown in Figure 4. The topologies outside of reticulation events were generally consistent with the species tree topology, although the specific relationships among *B. aurifer*, *B. bicolor* and *B. rowleyi* did vary. The three methods recovered different reticulate relationships, though several nuclear Middle American lineages, especially *B. aurifer*, *B. marchi* and *B. thalassinus*, were implicated in multiple methods. PHYLONET recovered a reticulation from *B. aurifer* (minor edge) to *B. thlassinus* (major edge) and from an ancestral nuclear Middle American lineage to the ancestor of *B. bicolor* and *B. rowleyi*. In contrast, SNaQ inferred reticulations from *B. aurifer* (minor edge) to the ancestor of *B. marchi* and *B. thalassinus* and a second, a more genetically limited transfer between *B. supraciliaris* (minor edge) and the ancestor of the montane *Bothriechis*.

For TREEMIX inference, we extracted 368 putatively independent SNPs (i.e. one SNP per locus) excluding loci for which there were missing taxa. The overall topology inferred from TREEMIX reflected the relationships recovered in phylogenetic analyses of the anchored loci, with the addition of a migration edge between *B. marchi* and the ancestor of *B. aurifer*, *B. bicolor* (Figure 4) and migration between *B.*
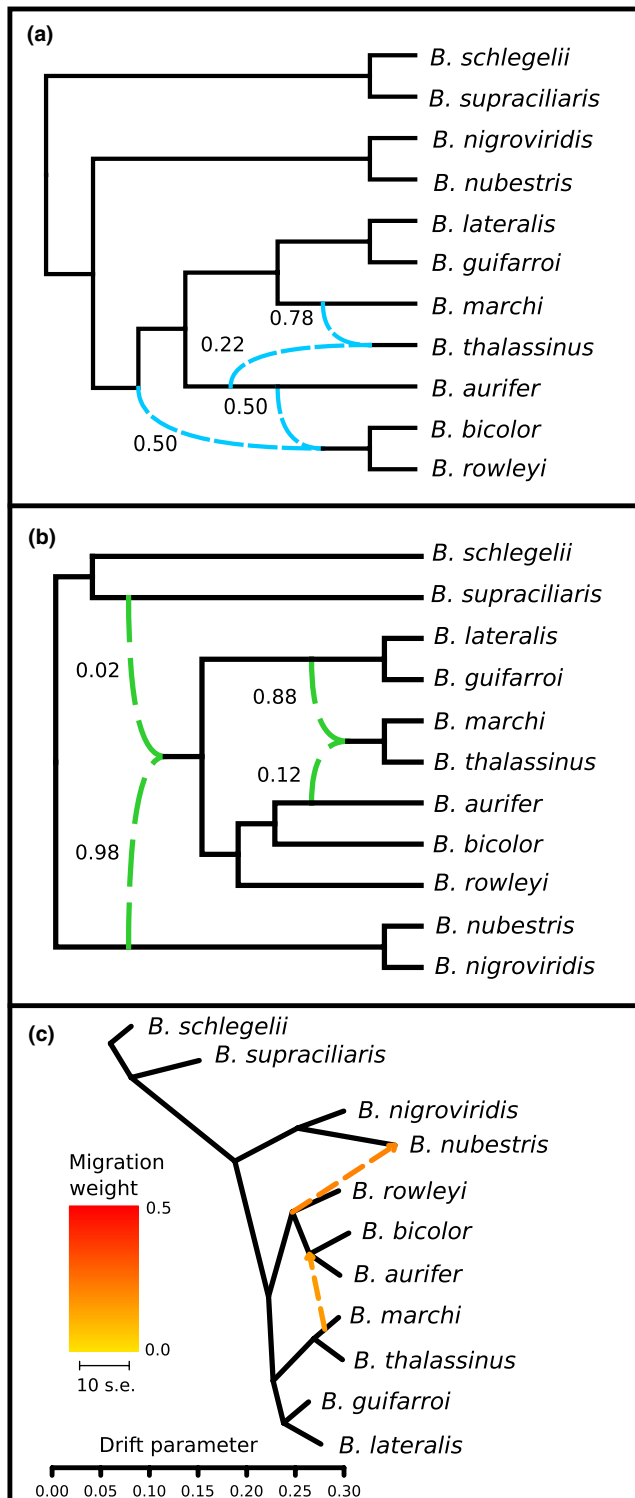
FIGURE 4 Top phylogenetic networks inferred for *Bothriechis* by (a) PHYLONET, (b) SNAQ, and (c) TREEMIX. Dashed edges represent reticulation among lineages. Numbers adjacent to reticulation edges show inheritance probabilities for PHYLONET and SNAQ. Color of reticulation edges in (c) indicate migration (i.e. geneflow) weights [Colour figure can be viewed at wileyonlinelibrary.com]

these tests limited ability to detect gene flow occurring before speciation events which was suggested in network analyses (see Tables S4 and S5 in Appendix S6). In contrast, *D*-statistics showed strong evidence for admixture among many population combinations, including several of the nuclear Middle American lineages identified in previous analyses (see Table S7 in Appendix S6).

4 | DISCUSSION

As genomic datasets have become increasingly available, there has been a corresponding rise in the identification and recognition of complex evolutionary histories (Arcila et al., 2017; Jeffroy et al., 2006; Thom et al., 2018). Our phylogenomic analysis of *Bothriechis* reveals reticulate evolution in nuclear Middle America leading to conflict in the phylogenetic placement of some lineages. Biogeographic dating and ancestral area reconstruction indicate that the taxa most often implicated were present in adjacent regions during the time when reticulation occurred. Finally, the inferred reticulations in the phylogeny resolve historical incongruences in the phylogeny of *Bothriechis*.

The prevailing hypothesis for the biogeographic history of *Bothriechis* has been the northern dispersal model suggested by mitochondrial data (Castoe et al., 2009; Daza et al., 2010), but our phylogenomic sampling shows that this model does not effectively explain the patterns of diversification and phylogenetic conflict in these data. In contrast, our biogeographic reconstruction suggests that the common ancestor of *Bothriechis* was likely widely distributed. The common ancestor of the "montane" *Bothriechis*, which includes all species outside of *B. schlegelii* and *B. supraciliaris*, likely ranged from the Isthmus of Panama to southern Mexico. Speciation within this group then occurred through a combination of sympatric and vicariant processes. The high-elevation species *B. nigroviridis* and *B. nubestris* became restricted to the cordilleras of Costa Rica and Panama, perhaps as a result of specialization for higher elevational distributions and niche partitioning with mid-elevation taxa (Figure 3). Speciation in nuclear Middle America appears to have occurred largely through vicariance, especially in the divergence of the montane Chortís block species. Here, tectonic activity (Rogers et al., 2007) and possible climatic fluctuations such as those associated with the final closure of the Isthmus of Panama (Lunt, Valdes, Haywood, & Rutt, 2008), likely promoted isolation and speciation while later allowing secondary contact and gene flow among lineages.

We find strong support for several general conclusions regarding gene flow in nuclear Middle America which likely involved at least *B. aurifer* and either *B. marchi*, *B. thalassinus*, or their common ancestor. Though the specifics of which lineages were involved and the extent of inferred genomic exchange varied among methods, gene flow among lineages in this region appears to have been substantial enough to cause the conflicting phylogenetic signals observed in this study and previous work (Taggart et al., 2001). While the variation in networks recovered by PHYLONET, SNAQ and TREEMIX is partially due to technical differences among methods, it likely also reflects limited or conflicting biological signal due to low numbers of informative sites or weak signal of introgression among sampled gene trees.

rowleyi and *B. nubestris*. Three-population and four-population tests did not show definitive admixture or gene flow among taxa, although this may reflect our usage of species rather than populations, or

In the latter case, improved sampling at the genomic level and/or population level may add clarity to the direction and magnitude of inferred reticulation. Regardless, for gene flow to occur, lineages must occupy (or have occupied) geographically proximate regions that would allow secondary contact (Burbrink & Gehara, 2018). Our ancestral area reconstruction, as well as the modern distributions of these taxa, place the lineages putatively involved in this reticulation in adjacent regions on either side of the Matagua–Polochic fault. Moreover, based on our fossil calibrated tree, this reticulation likely occurred between ~8–3 Ma. This time period corresponds to an interval of high volcanic and tectonic activity in the region; conditions which could lead to repeated instances of isolation and secondary contact (Rogers et al., 2007; Townsend, 2014). Regardless of the mechanisms promoting contact, the reticulate evolution observed in this group and the phylogenetic conflict it introduced underscores the importance of these processes to the *Bothriechis* phylogeny.

Hybridization can lead to adaptive introgression, wherein natural selection maintains introgressed alleles, which may allow for rapid adaptation in dynamic environments (Suarez-Gonzalez, Lexer, & Cronk, 2018). If adaptive introgression plays a role in the reticulate evolution identified in *Bothriechis*, identifying loci that have introgressed from across the genome and determining how these have impacted phenotypes would be informative for our understanding of adaptation and trait evolution. The addition of genetic variation attained through hybridization and introgression has also been proposed as an important driver of speciation (Abbott et al., 2013; Seehausen, 2004), even leading to adaptive radiations as seen in some systems (Grummer, Morando, Avila, Sites, & Leaché, 2018; Meier et al., 2017; Salzburger, Baric, & Sturmbauer, 2002). While this does not appear to be the case in *Bothriechis*, much of the genera's range is poorly sampled and future surveys of currently unsampled populations may lead to recognition of additional species and stronger support for the role of reticulate evolution in the diversification of this group. Unfortunately, due to the highly disjunct distributions of *Bothriechis* and the logistical challenges associated with reaching them, many species' ranges and populations remain under sampled (Campbell & Lamar, 2004; McCranie, 2011; Savage, 2002; Wilson & McCranie, 2004). This may be a promising avenue for future study, though it is beyond the scope of the current work.

Phylogenomic approaches have led to increased resolution in many taxonomic groups, especially when combined with explicit tests of complex or non-tree-like evolutionary processes (Burbrink & Gehara, 2018; Grummer et al., 2018; Thom et al., 2018). Dynamic geologic and climatic histories are often associated with diversification and speciation processes, but these conditions can also promote complex evolutionary interactions that make it difficult to infer a strictly bifurcating tree. The increasing appearance of approaches for identifying and testing for reticulate evolution and gene flow among lineages corresponds with a growing appreciation for the role of these processes in evolutionary biology and speciation. However, the ability to detect and characterize ancient hybridization events is highly dependent on the characteristics of a given dataset as well as the specific analytical methods used, each of which have their own strengths and weaknesses (see Appendix S7 for additional discussion on this topic). Complex histories can occur at any taxonomic level, are not apparent with every dataset, nor detectable with every analytical method making their true prevalence in the tree of life unclear. Though genomic resources can provide the raw materials for resolving complex evolutionary processes, explicitly testing alternative hypotheses using several approaches and multiple lines of evidence remains the best strategy for bringing these histories into focus.

DATA ACCESSIBILITY

Sequences used here are available on Genbank under the accession numbers in Appendix S1. Additional resources including sequences alignments, commands and input files for analyses are available on the DRYAD Digital Repository (https://doi.org/10.5061/dryad.7n42q35). Title: Data from: Reticulate evolution in Nuclear Middle America causes discordance in the phylogeny of palm-pitvipers (Viperidae: Bothriechis)
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BIOSKETCH

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Author contributions: A.J.M., C.L.P and F.G. designed the study; C.L.P., F.G. and H.Z. provided samples and data; F.G., A.R.L. and E.M.L. conducted the laboratory work and generated sequences; A.J.M. performed bioinformatic processing, analyses, drafted the manuscript and generated figures; A.J.M., A.R.L., C.L.P., E.M.L., F.G. and H.Z. reviewed and edited the manuscript.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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